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JUN 11 1954

VOLUME 12 NUMBER 6

NUTRITION REVIEWS

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June 1954

Special Article
Food Attitudes—A Part of Nutrition Education 161
Clinical Nutrition
Distribution of Body Fluid and Electrolytes in Heart Failure Due to Beriberi 162
The Nature of Bound Vitamin B_{12} in Blood Serum 164
Animal Nutrition
Influence of Roughages on Milk 165
Nutritional Applications of Antibiotics 166
Nutrition and Agriculture
Amino Acid Content of Green Leaf Forages and Meals 168
Effects of Maleic Hydrazide on Plant Growth Inhibition 169
Protein Quality in Cottonseed Meal 171
Experimental Nutrition
Comparison of Chemical and Biologic Activities of L-Ascorbic Acid and D-Isoascorbic Acid 172
Squalene, A Precursor of Cholesterol 174
The Nature of the *Lactobacillus bifidus* Factor 175
Glutathione and Ascorbic Acid in Liver Necrosis 177
Mannitol and Sorbitol as Dietary Constituents 178
Solvent Toxicity Relations with Protein and Vitamins A and E 179
The Influence of Magnesium on Protein Synthesis 181
Studies on Nitrogen Balance 182
Nutrition Experiments with Army Rations 184
Biochemical Effects of Vitamin B_6 Deficiency 186
Fructose Metabolism in the Liver 187
Hydrocephalus Associated with Deficiency of Vitamin A 189
Notes
Letters to the Editor 191
Composition of Yeast Protein 191
Changes in Peanut Composition During Roasting 192
Ferric Versus Ferrous Iron for Prevention of Hypochromic Anemia in Infants 192

Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.

For

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK 17, N. Y.

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Changes of address should be sent to The Nutrition Foundation.
Application for entry as second-class matter pending at the Post Office at Baltimore, Md.

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NUTRITION REVIEWS

VOL. 12 JUNE 1954 No. 6

FOOD ATTITUDES—A PART OF NUTRITION EDUCATION

The Department of Nutrition at the Harvard School of Public Health from the time of its organization has been interested in nutrition education. The department believes that the nutritional practices of large groups of people can be improved by helping children in elementary and secondary schools to develop desirable food habits. Consequently, the department, together with the communities of Newton, Massachusetts, Rutherford County, Tennessee, and Ascension Parish, Louisiana, has conducted intensive nutrition education studies in the schools.

In these studies children learn about and practice principles of good nutrition in school so that they may acquire better food practices and attitudes which will carry over into the home and adulthood, and which may eventually become the practices and attitudes of their offspring. Although nutrition education programs are making available to children nutritional information and opportunities to experience good food practices, less attention has been paid to developing general attitudes and a sense of values. Attitudes can have a lasting effect beyond the duration of the immediate program.

The individual's opinions about food and eating affect what he eats; therefore they affect his total well-being both directly and indirectly. One of the objectives of nutrition education is to encourage, implant, modify, or eliminate attitudes which govern the relationship between an individual and the food he eats, as well as his willingness to accept and apply nutritional information.

The dominant role that attitudes play in our thought, memory, and learning processes should not be overlooked. Facts help in the development of understandings and appreciations of food, food practices and attitudes, but attitudes that are already formed influence the acceptance and application of new knowledge and thereby control food behavior. An attitude acquired through any experience with food may be retained long after the student has forgotten the facts he learned. The individual tends to select, remember and use information which fits the attitudes he already has and to reject facts which conflict with his views. Studies have shown that students with favorable attitudes toward certain material learn it more readily than those whose attitudes are unfavorable. Attitudes should be considered both as basic to educational activities and as products of education.

For purposes of nutrition education a food attitude is here defined as an inclination to react a certain way toward food and situations involving food. This inclination may be based on an individual's cultural background, experiences, ideas, preconceived notions, convictions and prejudices about food.

Where do attitudes originate? They are acquired or learned. G. Allport ("Handbook of Social Psychology," edited by C. Murchison, *Clark University Press, Worcester, Mass. (1935)*) says that attitudes may develop in four ways: (1) from the accumulation of a large number of experiences over a long period of time; (2) from the splitting of a specific attitude from a more general one; (3) from an unusual, violent, or painful experience; and (4) by adoption from examples set by such attitude-molding mediums as parents, friends, teachers, movies, radio and television.

Individuals who have similar attitudes may differ according to the intensity with which they maintain them. This intensity

can range along a scale from strong agreement to strong disagreement with the idea that the attitude expresses. The midpoint of the scale is neutrality. A number of technics have been developed for the study of attitudes held by groups and individuals. Group measurement technics are suitable for the study of public attitudes while clinical interviews, psychoanalysis and other technics may be used to investigate subconscious and private attitudes.

Some of this knowledge about attitudes can play an important role in planning nutrition education programs. Educators realize that each individual has been exposed both in and out of school to many different beliefs, ideas, notions and convictions about food. Research in nutrition education should therefore direct some of its efforts toward the exploration of food attitudes. If educators knew more about their students' beliefs, ideas, notions and convictions about food, they would be better able to develop effective nutrition education programs.

The Department of Nutrition at the Harvard School of Public Health has already initiated a study to determine what food attitudes are prevalent among high school students and to measure the intensity with which these attitudes are held.

In order to make certain that the questionnaire which is being used to measure the food attitudes of high school students contained statements representative of those held by boys and girls of this age group a preliminary investigation was made. Students from two communities of different socioeconomic status were asked to write short unsigned essays on the subject, "What I Think and Feel About Food." A long list of statements which could be considered expressions of attitudes was accumulated from approximately 200 essays. After careful examination and editing of each statement, some of the attitudes which are of interest in nutrition education were assembled into an experimental questionnaire which should afford some measure of the attitudes of high school pupils toward food.

The questionnaire has been administered to approximately 2000 students. When the results become available it is hoped that these or modified technics will provide some information which will aid in planning nutrition education programs embracing not only the nutritional knowledge and food practices of students but also their interests, experiences and beliefs.

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DISTRIBUTION OF BODY FLUID AND ELECTROLYTES IN HEART FAILURE DUE TO BERIBERI

The cardiac musculature is particularly susceptible to deficiency in thiamine. Heart failure due to beriberi is of the high-output type (*Nutrition Reviews* **4**, *24* (*1946*)) and may be differentiated from cardiac decompensation of other causes. In the United States beriberi heart disease is observed most commonly in patients with a history of limited food intake associated with chronic alcoholism. The purpose of a recently published investigation was to study the shifts in distribution of body water and electrolytes in this disorder, as well as the changes in these which occurred with nutrient therapy (L. T. Iseri and co-workers, *Circulation* **9**, *247* (*1954*)).

Detailed metabolic study was undertaken of 3 patients with severe congestive heart failure due to beriberi. There was history of chronic alcoholism with poor dietary intake, and clinical manifestations of avitaminosis were observed in each patient. Histories and physical examination excluded pericardial, valvular and myocardial disease of other

origin. The congestive heart failure in these patients was characterized by edema, elevated venous pressure, increased pulse pressure, decreased circulation time, increased cardiac output and peripheral vasodilatation. Recovery occurred during periods of treatment by a regimen of bed rest and low salt and vitamin-rich diet with or without supplements of thiamine. Digitalis and mercurial diuretics were not used in treatment.

The cardiac output and intravascular and intracardiac pressures were determined by cardiac catheterization before and after therapy, as were specific renal function tests. In addition, balance studies for water, nitrogen, sodium, potassium and chloride were carried out during the recovery period. Intracellular and extracellular distribution of water and electrolytes was calculated by standard technics.

There was loss of body water during the period of treatment, as was to be anticipated during a period of recovery from cardiac decompensation. The total loss of water during periods of eight to twelve days of study in the 3 patients was 22.0, 17.7 and 5.5 kg. Most of this was derived from extracellular sources. The total extracellular water was calculated to be 34, 41 and 17.6 per cent of edematous body weight, respectively, in the 3 cases at the onset of treatment. This compares with 16 per cent of body weight in compensated individuals. It was calculated that loss of water from extracellular sites was 20.7, 19.5 and 2.3 kg. in the 3 individuals, these changes being listed in the same order as the other values given above. During the period of treatment there was loss of intracellular water in 2 of the cases, but a shift of water into this compartment occurred in the third patient.

During the period of dietary treatment and supplementation with thiamine there was loss of large quantities of sodium ion from the body. Total losses were 2620, 1740 and 500 milliequivalents (mEq) in the 3 patients. The outpouring of sodium in the urine occurred promptly with the provision of a highly nutritious diet of low salt content in 2 patients, but was delayed in one patient until supplements of 200 mg. of thiamine given intramuscularly three times a day and vitamin B complex given orally were provided. The sodium content of cells varied during the period of treatment. The total effect was one of decrease in intracellular sodium in 2 patients and a substantial increase in the third.

There was retention of potassium in each of the subjects during the period of dietary treatment, which was related to actual increase in intracellular potassium. When the quantity of osmotically active base as calculated at the beginning of the experimental period was compared with that at the end, there was indication of binding of osmotically active electrolyte in intracellular fluids.

The metabolic study of these patients during recovery from beriberi heart disease indicated movement of sodium, potassium and water between intracellular and extracellular fluid compartments so that there was increase in the total electrolyte in cells. It is presumed that the capacity of cells to take up additional electrolyte during recovery is dependent upon osmotic inactivation of intracellular base. These changes are in the same direction as those which occur in low-output heart failure (L. T. Iseri, A. J. Boyle, and G. B. Myers, *Am. Heart J.* **40,** *706 (1950)*; R. D. Squires, A. P. Crosley, and J. R. Elkinton, *Circulation* **4,** *868 (1951)*).

The authors point out that an increase in cellular osmolarity may lead to release of electrolytes from cells and the uptake of water, leading finally to the development of interstitial edema. Such an event in low-output failure may be attributed to primary cardiac insufficiency leading to accumulation of metabolites in tissue because of inadequate removal or impaired oxidation. The sequence of events in the high-output failure

of beriberi is quite different since it occurs in the presence of adequate or increased oxygen transport and since it may develop in the absence of myocardial failure. It is suggested that the cellular osmolarity in beriberi is increased through the accumulation of pyruvate and lactate in the tissues. This would represent the initial event in a cycle which then might lead to myocardial failure and reduced renal blood flow.

THE NATURE OF BOUND VITAMIN B_{12} IN BLOOD SERUM

Vitamin B_{12} is present in serum in free form and also in bound form. The bound form stimulates growth of the green alga *Euglena gracilis* only after heating at 100°C. for fifteen minutes, whereas the free form of the vitamin stimulates growth of the microorganism without prior heat treatment (G. I. M. Ross, *J. Clin. Path.* **5,** *250 (1952)*). D. L. Mollin and Ross (*Ibid.* **5,** *129 (1952)*) reported that patients with megaloblastic anemias which responded to vitamin B_{12} all exhibited low levels of serum-bound vitamin B_{12} prior to treatment. They suggested that the bound form of the vitamin represents the hematopoietically active fraction. D. L. Horrigan and R. W. Heinle (*J. Lab. Clin. Med.* **40,** *811 (1952)*) reported on a patient with anemia which responded to treatment with human plasma. During relapse this patient excreted the major part of a test dose of radioactive vitamin B_{12}; this excretion was markedly reduced when plasma was given. Horrigan and Heinle suggested that the anemia resulted from a vitamin B_{12} deficiency caused by a failure of proper binding of the vitamin in the serum.

These considerations all indicate the importance of bound vitamin B_{12} in serum, and the report of W. R. Pitney, M. F. Beard, and E. J. Van Loon (*J. Biol. Chem.* **207,** *143 (1954)*) concerning the nature of the bound form of the vitamin is of considerable interest. These workers used a combination of paper electrophoresis and microbiologic assay with *Euglena* in their experiments. A sample of serum was placed on the paper strip and subjected to the usual electrophoretic procedure. After development of the strips, the protein bands were detected by alcoholic bromphenol blue. The strips were then cut into sections which contained albumin, alpha-1, alpha-2, beta and gamma globulin. These sections were placed in separate test tubes which contained the basal medium for the organism and heated in a boiling water bath for fifteen minutes. The extracted vitamin B_{12} was then assayed by the *Euglena* assay. Under these conditions only the alpha-1 and alpha-2 globulin fractions contained significant quantities of vitamin B_{12}. It thus appears that the bound form of vitamin B_{12} is a complex of the vitamin and alpha globulin of serum.

Other experiments were conducted to determine the ability of serum to bind vitamin B_{12}. Increasing quantities of crystalline vitamin B_{12}, ranging from 50 to 1000 micrograms per milliliter, were added to normal serum and after a two-hour incubation at 37°C. the contents of free and bound vitamin B_{12} were determined. Previous experiments with a large number of serums had shown that normal serum contained only traces of free vitamin B_{12}, the major portion of the vitamin being present in the bound form. The quantity of bound vitamin B_{12} present in the serum after incubation was found to increase in response to the *in vitro* addition of the vitamin as the amount added was increased to approximately 200 micrograms per milliliter. The addition of vitamin B_{12} beyond this value did not result in an increased concentration of the bound form of the vitamin. In contrast, the amount of free vitamin B_{12} present after incubation was found to increase in response to the *in vitro* addition of the vitamin throughout

the range studied. It appears that the ability of serum to bind vitamin B_{12} is limited and this limit probably represents saturation of the alpha globulin fraction which binds the vitamin.

Further study of the binding of vitamin B_{12} in serum seems indicated in view of the observations suggesting that the bound form may be closely related to the hematopoietically active material. It will be of particular interest to determine the relationship, if any, of this bound form of the vitamin to the intrinsic factor of pernicious anemia.

INFLUENCE OF ROUGHAGES ON MILK

Workers at Cornell University have demonstrated that the ability of cow's milk to resist reactions that produce oxidized flavors is in part dependent upon the tocopherol content of the milk fat. It was further found that the level of milk carotenoids was positively correlated with the level of milk tocopherols (*Nutrition Reviews* **9,** *118 (1951)*). In a recent experiment these workers have investigated the antioxidant properties of milk as influenced by roughages harvested at different stages of maturity (V. N. Krukovsky and co-workers, *J. Dairy Sci.* **37,** *1 (1954)*).

Twelve Holstein cows served as test animals. They were used to test four roughages in four five-week feeding trials. The four roughages were as follows: early-cut silage, timothy heads in boot; early-cut, barn-cured hay, timothy headed but prebloom; late silage; and field-cured hay, cut with the timothy in full bloom. The roughages were a mixture of 50 per cent of clover, 25 to 30 per cent of grass (predominantly timothy), 5 to 10 per cent of alfalfa, and the remainder was weeds. The cows were fed the roughage ad libitum and received an average of 10.4 pounds of grain concentrate mixture per head daily.

The stability of the milk to oxidized flavors was determined organoleptically over a ten-day holding period at 0 to 5°C. in the presence of 0.1, 0.5, and 1.0 mg. of copper per liter.

Stage of harvesting and mode of processing had a striking influence on the total tocopherols and carotene content of the roughage. For example, the early-cut silage averaged 470 micrograms of total tocopherols per gram, whereas the late field-cured hay averaged only 107 micrograms per gram (dry matter basis). Carotene values were even more strikingly related to harvesting and processing. The early-cut silage contained 422 micrograms per gram, whereas the late field-cured hay contained only 16 micrograms per gram.

On the average the milk fat produced on the early-cut silage was highest in tocopherols, total vitamin A value and carotenoids; the milk fat produced on late-cut field-cured hay was lowest. Differences in the milk levels of the vitamins, as affected by roughage levels of the vitamins, were highly significant.

Only 0.28 per cent of the carotene in the high-carotene, high-tocopherol roughage (early-cut silage) was recovered in the milk as vitamin A or carotenoids. In contrast, 4.59 per cent of the carotene in the low-carotene roughage (late-cut, field-cured hay) was recovered in the milk as total vitamin A activity. In the high-carotene roughage the ratio of tocopherol to carotene was 1:1, whereas in the low-carotene roughage this ratio was about 10:1. These results are indicative of increased efficiency of carotene utilization with increased tocopherol-to-carotene ratio in the roughage fed.

Milk produced on late-cut silage retained its ability to resist oxidized flavors when stored in the presence of copper for ten days. In contrast, the resistance of milk to oxidized flavors was appreciably reduced

when the early-cut silage, barn-cured hay or field-cured hay were fed. Fifty-three per cent of these became unpalatable after ten days of storage. The resistance of milk to the development of oxidized flavors was therefore not directly correlated with milk tocopherol level alone. The early-cut silage produced milk with an average of 3090 micrograms of tocopherol per 100 g. of milk fat, whereas the late-cut silage, which produced more stable milk, contained an average of 2614 micrograms of tocopherol per 100 g. of milk fat.

These studies reveal that the biochemical properties of milk which relate to its stability can be quite markedly influenced by the type of ration fed to the cow. Roughages appear to contain variable amounts of substances in addition to tocopherols that extend their protective influences.

NUTRITIONAL APPLICATIONS OF ANTIBIOTICS

Since 1950 many reports have appeared describing beneficial effects of feeding antibiotics to farm animals as feed supplements. These antibiotic supplements are already the basis of an important commercial business in feeds for young pigs and chicks. Calf feeds are beginning to contain them also. These and other applications to agricultural and food industries are described in an editorial (*J. Agr. Food Chem.* **1,** *1096* (*1953*)).

The antibiotics most widely used for growth-stimulating effects are aureomycin, terramycin, diamine penicillin, procaine penicillin, and bacitracin. They have a dual action, being used at 5 to 20 parts per million (p.p.m.) as growth stimulants, or at 100 to 200 p.p.m. as therapeutic agents. The control of nonspecific diseases is accepted as a factor in growth stimulation, which is often related in extent to the subclinical disease level among animals that have a high incidence of such infections. Growth stimulation may be accounted for by suppression of microorganisms that compete with the animal for food supply or which tax the normal metabolism by disease, or by suppression of organisms of the gut which interfere with absorption of nutrients.

Use of radioactive thiamine indicates that feeding of antibiotics results in greater synthesis of vitamin B_1 in the intestinal tract. In the case of swine, bacteriologic examination of the feces of the animals fed antibiotics showed an increase over the controls in the population of the fungus *Aspergillus flavus*. Feed containing cultures of this fungus produced a growth response, but gave no indication of suppressed subclinical disease or that the fungus could replace antibiotics in all growth stimulation.

Many aspects of antibiotic feeding have been investigated since the first reports of the increased growth rates which may occur. Results on ruminants have been summarized by J. T. Reid, R. G. Warner, and J. K. Loosli (*J. Agr. Food Chem.* **2,** *186* (*1954*)). Addition of 10 to 100 mg. daily of aureomycin or terramycin to the ration of young calves (3 to 116 days old) resulted in body weight gains which were 10 to 40 per cent greater than those of calves consuming unsupplemented diets. The growth promotion is greater during very early life, especially during the first four weeks of life, than it is later. When 6 month old calves not previously fed these supplements have antibiotics added to their diet, a transitory growth stimulation occurs during a period of about six weeks. A large proportion of the gain is of muscle and fat rather than of skeleton, and the size of the growth response is largely the result of increased feed intakes rather than increased efficiency of feed use. Thus the importance of antibiotics in calf nutrition seems to depend on the ultimate disposition of the

calf, since the control animals not given antibiotics later attain a size equal to that of the animals which grew more rapidly in weight at an earlier age. Aureomycin stimulates growth up to 6 months of age and terramycin stimulates it up to 2 or 3 months of age. Animals that had been fed antibiotics from birth to the age of 3 years showed no detrimental effects on reproduction or milk production.

One pronounced beneficial effect that most workers have noted in calves fed antibiotics is the prevention of scours (a disease of the digestive tract commonly found in calves and more prevalent under unsanitary conditions), and better control of respiratory ailments. These benefits result in a sleeker hair coat, better physical appearance and greater general thriftiness. Moreover, high morbidity and mortality among young calves is greatly reduced by such feed supplements. This is the principal benefit and is especially important for production of veal calves.

The two antibiotics discussed above stimulate the appetite, while penicillin depresses feed consumption. Large doses of aureomycin depress the digestibility of crude fiber and the retention of nitrogen in older animals. In young calves these antibiotics apparently do not change the total number or kinds of bacteria in rumen ingesta, feces, or ingesta from the colon. Thus age of the animal has a very significant effect on the microflora of the intestinal tract in relation to antibiotics. More work seems necessary to develop more information about effects on vitamin synthesis and other effects in the digestive tract.

An obvious problem is the antibiotic carry-over from feed into animal products used for human consumption. Aureomycin can be detected in concentrations as low as 1 to 5 parts per 100 million, while others require higher levels for detection. Aureomycin fed at growth-stimulating levels could not be found as residue in the meat. At ten times higher levels, it could be detected in chicken flesh. The lowest feeding level producing detectable carry-over in pigs was 200 p.p.m. At a level of 1000 p.p.m. carry-over is detectable in most animal tissues; however, even here withdrawal of the antibiotic from the diet for several days allowed for clearance of the substance from the tissues. Aureomycin in meat is also destroyed by cooking. This situation thus appears favorable for meat, but may be different for eggs and milk which are not always cooked before eating. However, in lactating cows, no nutritional effect was evident and aureomycin was not transmitted to the milk nor did it inhibit the activity of cheese starter, which is a sensitive test for this antibiotic.

Limited data indicate no effect upon the rate of gain of beef cattle with antibiotic supplements in their fattening rations. Studies on lambs are inconclusive to date, although no outstanding benefit seems obvious.

It appears then that such feed supplements will have an increasing role in animal nutrition and consequently will affect human viewpoints as well. Economically, supplementation pays well because it improves feed efficiency up to 12 per cent with pigs. A saving of 5 per cent is average under average feed-lot conditions. The response is in proportion to the disease level; greatest response is obtained by addition to the deficient rations of animals with a high subclinical disease level. Disease-free animals do not respond to antibiotic feeding. Antibiotics do not replace sanitation, but do make profitable production possible under high disease-level conditions.

The control of plant diseases through systemic treatments with antibiotics is also entering the picture. With food plants this may be of great importance to human nutrition. Dramatic results have been reported against fireblight, a disease of fruit trees which caused the eradication of many pear and apple orchards of the southeastern United States. Streptomycin and terramycin

were applied as sprays. Encouraging results were also obtained for bean diseases. Actidione is in commercial use. Much more research will be necessary, and possible effects on human food must be constantly checked with each new development.

AMINO ACID CONTENT OF GREEN LEAF FORAGES AND MEALS

In considering vegetable waste utilization, the large quantities of leaves available during the harvest of certain crops present a possibility for development and use in stock feed. Leaf meals are high in protein. The amino acid studies of E. G. Kelley and R. R. Baum (*J. Agr. Food Chem.* **1,** *680 (1953)*) are of interest in this regard. These workers applied several analytic technics to the extraction and determination of protein and 10 constituent amino acids in leaf meals from beets, broccoli, carrots, celery, corn, kale, lima beans, peas, rhubarb, spinach and turnips.

Meals from these sources contained from 17 to 41 per cent crude protein. Individual amino acids exhibited ranges of 50 to 300 mg. per cent. These vegetable leaf meals are not concentrated sources of any of the 10 amino acids studied, but they do contain a mixture which would make them useful as supplementary sources of protein in animal and poultry diets. Broccoli leaf meal has been shown to serve well in high-energy poultry diets when combined with soybean meal as the source of protein.

In the southwest winter wheat belt the common cereals are widely used as pasture for livestock. Their foliage has a high nitrogen content, most of it existing as combined nitrogen in organic forms. Contents of specific amino acids in clippings from leafage of representative cereals have been investigated by E. Reber and R. MacVicar (*Agronomy J.* **45,** *17 (1953)*). Short plants 3 to 4 inches high were analyzed by microbiologic assay methods.

Leucine was found to the extent of 5.4 to 7.8 per cent in the bulk protein. The clippings resemble total proteins of muscle and whole egg in this respect. The bulk protein contains more leucine than most plant seeds. Isoleucine and valine followed a similar pattern. Threonine (3.6 to 5.9 per cent) was more abundant than in seed proteins but methionine was less abundant. The lysine concentration resembled that characteristic of most animal tissue proteins. This relatively high content makes such clippings good supplements for nonruminant animals, such as swine, for which rye has long been recognized as good pasture. Glutamic acid (plus glutamine) was the most abundant amino acid. Phenylalanine concentration was characteristic of other leaf proteins. Tryptophan occurred to only about one-half the content of most animal tissues. This is a serious limitation in the use of these grasses for supplementation of the concentrate portion of the ration.

Entire plants were analyzed at different stages from early spring growth to the late dough stage. Most amino acids showed a distinct minimum at the early jointing stage. All the cereals (rye, barley, oats, and two wheat varieties) showed a uniform pattern of amino acid distribution. Lysine and threonine decreased rapidly after early heading, while isoleucine and glutamic acid increased during head development. It is clear that pasture of young leafy growth would provide a better balance of amino acids to the livestock.

Such studies are also of direct importance to man because young cereal clippings have been suggested as high nutrient supplements in the diet. Alfalfa has likewise been suggested, though not so widely used. This plant was studied by V. L. Singleton, E. T. Mertz, and R. L. Davis (*Agronomy J.* **44,** *346 (1952)*), who carefully analyzed hydrolysates of fresh plants

(leaves plus stems, as usually cut for hay) by microbiologic methods for 18 amino acids.

Selections were made from the alfalfa breeding plots of Purdue University. One hundred of the most vigorous clones formed a composite sample for analysis and results were compared with those for Ranger alfalfa seed. The most abundant amino acids in the composite were alanine, aspartic acid, glutamic acid, leucine, proline, threonine and valine. On the percentage basis, all acids except tryptophan were present at higher levels in the seed than in the forage. Compared on the basis of content per gram of total nitrogen, the seed proteins contained more arginine, cystine, glutamic acid and histidine, and less alanine, aspartic acid and tryptophan than the proteins of the forage. The methionine contents were equal.

The amino acid composition of alfalfa forage was compared with that of Ladino clover for two consecutive seasons. The greatest difference was for 3 dispensable amino acids, cystine, proline, and tyrosine—even here, maximum differences were around 30 per cent. Assuming equal digestibility, these two plants would seem equally nutritious on the protein basis.

One hundred clones of alfalfa were separately analyzed for methionine, which ranged from 0.17 to 0.28 per cent or from 72 to 96 mg. per gram of total nitrogen. Selections both high and low in methionine were found in every variety. These variations do not appear very favorable for breeding alfalfa plants with higher methionine content. However, the fairly high content of so many amino acids speaks well for the nutritive value of alfalfa, particularly the leaves, where most of the protein is found.

EFFECTS OF MALEIC HYDRAZIDE ON PLANT GROWTH INHIBITION

Maleic hydrazide produces interesting responses when applied to plants, in that it inhibits the development of meristem tissue and thus stops growth. The mechanism of cell division appears to be the primary process affected. Since plant tumors represent "wild growth" somewhat analogous to cancer in animals and man, it is important to compare physiologic responses of both normal meristem tissue and tumor tissue to application of maleic hydrazide. This was done by R. M. Klein and D. T. Klein (*Am. J. Bot.* **39,** *727 (1952)*), who used tomato plants with crown-gall tumors, which are caused by a bacterial infection.

Applications of the chemical were made five days before and after inoculation with the crown-gall bacteria. It had no effect on the processes leading to initiation of crown-gall tumors; the growth of tumors, as measured by weight or size, was independent of the suppressive influence on the development of the host plant. Both the chemical and the tumor retarded plant development and their effects were additive. In concentrations up to 1000 parts per million (p.p.m.), maleic hydrazide did not affect the respiration of normal mature tomato stem or tumor tissue. It did inhibit the respiration of the bacteria but did not prevent multiplication of the bacteria. It definitely inhibits growth and respiration of active meristematic tissues. Thus physiologic differences are indicated between normal meristematic and tumor tissues, both of which consist of rapidly multiplying cells.

The rate of deterioration of fruits in storage is closely correlated with their rate of respiration. F. W. Southwick and W. H. Lachman (*Proc. Am. Soc. Hort. Sci.* **61,** *388 (1953)*) investigated the possibility of increasing the storage life of tomato fruits by application of maleic hydrazide, because of its possible inhibition of respiration. Water containing various concentrations of maleic hydrazide (diethanolamine salt) was

applied to the stem end of the fruit and after permitting absorption for one hour, carbon dioxide evolution was measured at 74°F. The absorption of water alone tended to hasten respiration and ripening. Maleic hydrazide at concentrations from 100 to 10,000 p.p.m. was slightly inhibitory (seldom more than 15 per cent) to these processes, for it eliminated the stimulatory effect of water absorption.

Attention has recently been drawn to the control of sprouting in stored potatoes by maleic hydrazide. This could result in reducing losses from sprouting in storage, markets, and in the household. Effects on quality are also important. Seven varieties grown in New York state were tested by E. J. Kennedy and O. Smith (*Proc. Am. Soc. Hort. Sci.* **61,** *395* (*1953*)), who sprayed potato plants in the field with from ¾ to 3 pounds of the diethanolamine salt per acre at several different dates prior to maturity. The potato crop was then stored at two temperatures, 40° and 50°F. Following a storage period, exhaustive tests were made for quality; the inhibition of sprouting by the maleic hydrazide was striking. Increasing rates of application reduced sprout growth and total weight loss in storage and increased the number of active eyes in tubers stored at 50°F. Such tubers with reduced sprouting are not suitable for planting because a poor stand is obtained. In tubers stored at 50°F. maleic hydrazide had no effect on specific gravity, after-peeling blackening, reducing-sugar content after storage, or on color or yield of potato chips. It also did not affect the black-spot response when tubers were stored at 40°F. (a temperature too low to develop much sprouting).

The practice of spraying apple trees with plant hormones (auxins) to check preharvest fruit abscission (fruit drop) is now widespread. Some auxins, however, have the disadvantage of causing increased respiration of certain varieties, resulting in shortened storage life. The possibility of minimizing this ripening effect by spraying maleic hydrazide with the auxin was studied by R. M. Smock, L. J. Edgerton, and M. B. Hoffman (*Proc. Am. Soc. Hort. Sci.* **60,** *184* (*1952*)). They found that 3 different auxins when applied alone stimulated both softening and respiration. Maleic hydrazide applied prior to harvest consistently retarded the softening rate of fruit on the tree and during a holding period at 74°F. after harvest. This effect varied from complete elimination of the ripening effect to a reduction of 50 per cent. Relative concentration of auxins and maleic hydrazide complicate the antagonistic relationships. Maleic hydrazide often checked the respiration rate during storage, but did not always do so. Perhaps this substance may influence some other physiologic process besides respiration. Maleic hydrazide did not interfere with "drop control" by the plant hormones, although when used alone it reduced abscission by 20 per cent, probably as a result of retarded ripening.

Since late bloom in the Valencia orange tree is correlated with large fruit size, a study on the effect of maleic hydrazide on orange and grapefruit trees was undertaken by L. C. Erickson, B. L. Brannaman, H. Z. Hield, and N. D. Miller (*Bot. Gaz.* **114,** *122* (*1952*)). The spray was effective in delaying the bloom of Valencia oranges, but the mean fruit size of the following crop was reduced rather than increased. No effects were noted in the quality of fruit from sprayed grapefruit trees. In the Washington Navel orange, the spray caused a reduction in fruit size, but increased the number of fruits per tree. When trees were sprayed at bloom, there were severe modifications in the fruit in the form of thickened peel and greatly reduced development of juice vesicles, especially in the smaller fruit. Off-flavor was also detected. When sprayed several weeks after bloom, no effects on the quality of juice were noted.

Some confusion exists in the literature regarding the effects of maleic hydrazide on starch metabolism. Treated plants have

often been reported to contain unusual amounts of sucrose, suggesting that starch synthesis might be interfered with by maleic hydrazide. V. A. Greulach (*Bot. Gaz.* **114,** *480 (1953)*) studied beans and tomatoes by comparison of both attached and detached leaves in relation to photosynthesis, respiration and starch content. His results show that maleic hydrazide does not block either the starch breakdown process or starch synthesis in these plants.

Work by D. S. Mikkelsen, R. B. Griffith, and D. Ririe (*Agronomy J.* **44,** *533 (1952)*) on sugar beets indicates that maleic hydrazide may offer an effective means of regulating sugar beet maturity. In the treatments tried, sucrose content, root yield per acre, and sucrose yield per acre were all increased by maleic hydrazide sprays. Stages of physiologic maturity and time of harvest are important factors. It appears important that mitosis be stopped but that photosynthesis and conduction facilities within the plant not be disturbed. This problem requires more study, but offers great commercial potentialities.

A powerful agent has been found in maleic hydrazide for control of plant cell multiplication. Application of this material under controlled and well-understood conditions may result in many more useful technics in improving and conserving our food supply. Moreover, it, or products formed from it, may become rather consistent constituents of many of our foods, since it is absorbed and translocated throughout plant tissues. Wide use in agricultural practice will necessitate examination of the possible effects of maleic hydrazide on animal nutrition and growth.

PROTEIN QUALITY IN COTTONSEED MEAL

Cottonseed meal, the residue left after oil extraction, is an important constituent of livestock feed, particularly for swine and poultry. Cooking of the kernels, prior to pressing to remove oil, is a general practice to improve quality of the oil and to bind gossypol and associated pigments. Concomitant with these advantages of cooking are two disadvantages, protein denaturation and loss of thiamine. Nutritional value to livestock is influenced by availability of protein, amount of free gossypol left, and amount of thiamine. Reaction of amino acids with sugars to form complexes resistant to enzyme action may also be important in such heat processing. These factors have been studied in relation to different processing methods by W. A. Pons, M. D. Murray, M. F. H. Le Blanc, and L. E. Castillon (*J. Am. Oil Chem. Soc.* **30,** *128 (1953)*).

Products from five mills were studied; two used the hydraulic press and three the screw press. Cooking times and temperatures varied. About 90 per cent of the oil in the raw kernels is extracted, leaving 10 per cent in the meal, which brings the oil content of the meal to around 5 per cent. It is considered that free gossypol should be reduced to 0.03 per cent or less in meals used for livestock feeds. In general, hydraulic-pressed meals are higher in free gossypol than screw-pressed meals. However, by proper cooking conditions, one hydraulic mill produced meals with a content of 0.042 per cent, which approaches the values of 0.034 and 0.038 attained by two of the screw-press mills. Screw-pressed oils contain several times as much gossypol as hydraulic-pressed oils.

Nitrogen solubility is reduced more in the screw-pressed meals due to higher temperatures developed in the press. These values averaged one-half to one-third the values for meals from the hydraulic presses. Thiamine contents of around 30 parts per million (p.p.m.) in the raw kernels were reduced from 40 to 80 per cent, the lower

reduction occurring in the hydraulic-pressed meals. This is definitely associated with cooking conditions.

The value of meal as a protein supplement is influenced by the extent of damage to protein during processing. Overheating during processing reduces protein efficiency (grams gained per gram of protein consumed) as measured by growth tests. In general, meals having high nitrogen solubility and thiamine content also have high nutritive value, as measured by protein efficiency. Electrophoretic measurements on buffer extracts of cottonseed meals of different nutritive value (different history of heat treatment) were reported by M. L. Karon, M. E. Adams, and A. M. Altschul (*J. Agr. Food Chem.* **1,** *314 (1953)*).

Two series of commercial meals were studied which had been subjected to differing heat treatments (either in temperature or time of heating) by the screw-press method. For comparison a laboratory solvent-extracted meal was studied in which heat damage was minimized. Protein solutions were analyzed in the Tiselius moving-boundary electrophoresis apparatus. Meals were also analyzed for thiamine content and were fed to chicks and rats to determine protein efficiencies.

All commercial meals showed greatly reduced nitrogen solubility, thiamine values and protein efficiencies in comparison with the laboratory solvent-extracted meal. In any series, the more severe the heat treatment, the greater was the reduction in all these values. Electrophoretic data revealed that two major components changed with processing conditions. As heat treatments became more severe, these components were gradually replaced by a single component. A third fast-moving minor component was found in the commercial meals but not in the laboratory meal. Some correlations could be made between electrophoretic results and nutritive value, but electrophoretic patterns are obtained only on soluble proteins whereas nutritive value is dependent on entire protein ingested by the animal. As heat damage is increased, the percentage of total protein contributing to the electrophoretic pattern decreases, as shown by decrease in area of the pattern, which parallels the decrease in nitrogen solubility. Electrophoretic analysis provides information about the condition of the soluble protein components, degree of resolution, and presence of new components, information not revealed by solubility measurements. If means could be developed to increase the solubility of heat-damaged protein components, this approach could be of greater usefulness.

COMPARISON OF CHEMICAL AND BIOLOGIC ACTIVITIES OF L-ASCORBIC ACID AND D-ISOASCORBIC ACID

The mode of action of ascorbic acid in physiologic processes has not been fully ascertained. Ascorbic acid has been shown to have a role in the oxidation of L-tyrosine and it has been claimed that the vitamin functions as a coenzyme in one step of this reaction, the oxidation of para-hydroxyphenylpyruvic acid (R. R. Sealock, R. L. Goodland, W. N. Sumerwell, and J. M. Brierly, *J. Biol. Chem.* **196,** *761 (1952)*); *Nutrition Reviews* **11,** *17 (1953)*). However, a number of other compounds of related structure restored the activity of this enzyme after removal of ascorbic acid by dialysis. The active compounds were D-isoascorbic acid, D-glucoascorbic acid and reductone. Each of these compounds, like ascorbic acid, contains an enediol linkage. However, none has been demonstrated to be active in prevention or alleviation of the manifestations of scurvy. Therefore, the function of ascorbic acid as a coenzyme in

tyrosine oxidation cannot be considered to be fundamental in the causation of scurvy.

K. Guggenheim, J. Leibowitz, and D. Rakover (*Biochem. J.* **55,** *388 (1953)*) have recently published the results of investigations in which the chemical and biologic activity of ascorbic acid is compared with that of D-isoascorbic acid. These observers had long been interested in the effect of ascorbic acid in the detoxification of potassium cyanide, phenol, and tetanus toxin. When ascorbic acid or isoascorbic acid was added to solutions of phenol, changes in optical rotation were induced which were of the same order of magnitude for both substances. Similarly, it was found by determination of freezing point depressions that addition of either ascorbic acid or isoascorbic acid to solutions of phenol or potassium cyanide was associated with a decrease in molecular concentration, affording evidence of partial combination of these compounds to form condensation or addition products. The chemical affinities of ascorbic acid or isoascorbic acid for these toxins in solution having been demonstrated by physical measurements, the investigators then attempted an evaluation of detoxification by biologic procedures.

Potassium cyanide in concentrations of from 0.005 to 0.02 molar in nutrient broth was shown to inhibit markedly the growth of *Escherichia coli*. Growth was measured by determination of the turbidity of the medium after incubation for twenty-four hours. Addition of 0.01 molar ascorbic acid or 0.01 molar isoascorbic acid to the medium just prior to inoculation with the bacteria did not counteract the effect of potassium cyanide. However, if solutions of potassium cyanide and ascorbic acid or isoascorbic acid were mixed and allowed to stand for twenty-four hours at room temperature prior to their addition to the nutrient broth, there was a marked decrease in the toxic activity of the potassium cyanide, growth of the bacteria proceeding in some instances with little or no inhibition. There was no significant difference between the detoxifying activity of ascorbic acid and isoascorbic acid.

The toxic effect of phenol was studied in young rats. Injection by a subcutaneous route of 50 mg. of phenol per 100 g. of body weight caused death in all of a group of 22 rats. When equimolecular quantities of ascorbic acid or isoascorbic acid were added to the phenol solution and incubated for four hours at 40°C. before injection, there was a significant depression of the lethal effect of phenol. There were only 6 deaths in 22 animals to which the phenol-ascorbic acid solution was administered and 12 deaths in 22 animals receiving the phenol-isoascorbic acid mixture. The differences in death rates between the groups receiving ascorbic acid and isoascorbic acid were not adjudged to be significant.

In another experiment it was demonstrated that ascorbic acid and isoascorbic acid were equally effective in detoxification of tetanus toxin. The toxin was prepared by growing *Clostridium tetani* in Tarozzi broth at 37°C. for ten days. The toxin-containing supernatant was separated by filtration and centrifugation. Injection of 0.1 ml. of a 1:100 dilution of this material per 10 g. body weight in 10 mice caused death in all animals within six days. Incubation of the toxin with solutions of ascorbic acid or isoascorbic acid for twenty-four hours at a temperature of 37°C. prior to inoculation totally prevented deaths in other groups of 10 mice. In preparation of the toxin for this injection equal quantities of the diluted toxic broth were combined with solutions containing 1 mg. per milliliter of ascorbic acid or isoascorbic acid. The experiment was repeated using less dilution of the original broth, 1:8. In this instance the detoxification by ascorbic acid and isoascorbic acid was not total, with 20 and 21 animals dying within six days in respective groups of 30 rats.

The observations of other investigators have demonstrated that ascorbic acid

stimulates or promotes the formation of citrovorum factor from folacin (*Nutrition Reviews* **10,** *216 (1952)*). Guggenheim and his associates (*loc. cit.*) tested the effects of isoascorbic acid in this system. To do this the quantity of citrovorum factor excreted per day by rats on a purified diet without added folacin was determined. A biologic assay utilizing *Leuconostoc citrovorum* was the method of determination. The mean daily rate of excretion of citrovorum factor was about 3 micrograms for the animals on the basal diet for six days. This was increased to 13 micrograms when 1.0 mg. of folacin was injected intraperitoneally daily. In another period the rats were given 17.6 mg. of ascorbic acid in addition to the folacin. This resulted in a marked increase in the quantity of citrovorum factor excreted and a mean rate of 142 micrograms daily. Substitution of a similar quantity of isoascorbic acid for the ascorbic acid also increased the rate of citrovorum factor excretion to a value of 153 micrograms. Therefore, in this biologic function as well as in the detoxification systems studied, isoascorbic acid proved to be as active as ascorbic acid.

The data accumulated by these investigators show that isoascorbic acid possesses many properties identical with those of ascorbic acid. Moreover, other reports indicate that isoascorbic acid can effectively replace ascorbic acid in promotion of the oxidation of tyrosine and parahydroxyphenylpyruvic acid (Sealock *et al.*, *loc. cit.*; W. E. Knox and M. Le May-Knox, *Biochem. J.* **49,** *686 (1951)*). Since isoascorbic acid possesses only one twentieth of the activity of ascorbic acid in the prevention of scurvy (V. Demole, *Ibid.* **28,** *770 (1934)*), it would seem highly unlikely that the processes of detoxification, conversion of folacin to citrovorum factor, and oxidation of tyrosine are fundamental in the vitamin activity of ascorbic acid.

SQUALENE, A PRECURSOR OF CHOLESTEROL

Very little is known about cholesterol synthesis in the body, outside of the fact that it can take place from acetate as the raw material and is influenced by a number of metabolic conditions (*Nutrition Reviews* **10,** *345 (1952)*). The problem of finding intermediaries in the pathway from acetate to cholesterol is therefore one of particular importance. H. J. Channon (*Biochem. J.* **20,** *400 (1926)*), and Channon and G. R. Tristram (*Ibid.* **31,** *738 (1937)*) reported that the feeding of squalene to rats resulted in an increased cholesterol content of the liver. Squalene is an unsaturated hydrocarbon, $C_{30}H_{50}$, which forms a large proportion of the liver oil of certain fish (shark, the subclass *Elasmobranchii* generally), and also occurs in yeast. It is made up of six isoprene residues and contains six isolated double bonds. Its constitution has been arrived at by synthesis (P. Karrer and A. Helfenstein, *Helvet. chim. acta* **14,** *78 (1931)*).

These observations led two groups, that of Tomkins and Chaikoff in California, and that of Bloch in Chicago, to study the possible conversion of squalene to cholesterol, using radioactive carbon as a tracer. P. A. Srere (Dissertation, *University of California (1951)*, summarized by G. M. Tomkins, W. G. Dauben, H. Sheppard, and I. L. Chaikoff, *J. Biol. Chem.* **202,** *487 (1953)*) studied the conversion of acetate-C^{14} to cholesterol in liver slices prepared from rats fed a diet containing 1 per cent squalene. The livers from the squalene-fed rats showed a marked depression in their incorporation of the C^{14} into cholesterol but not in incorporation into carbon dioxide. This depression appeared compatible with the idea that the fed squalene had diluted a metabolic

pool (of squalene and compounds metabolically related). As a result of these findings Dauben and H. L. Bradlow (*J. Am. Chem. Soc.* **74,** *5204* (*1952*)) synthesized C^{14}-labeled squalene. The labeled squalene was administered to rats and incubated with liver slices. Surprisingly, no incorporation into cholesterol, either *in vivo* or *in vitro*, could be detected. Meanwhile, however, R. G. Langdon and K. Bloch (*Ibid.* **74,** *1869* (*1952*)) reported that the C^{14} of *biologically* prepared C^{14}-squalene (prepared from the nonsaponifiable fraction of the liver fat of rats fed C^{14}-carboxyl acetate) was incorporated into cholesterol *in vivo*.

A clue to the explanation of this apparent contradiction was obtained when it was observed that the infrared spectrums of the natural and the synthetic compounds differed (W. G. Dauben *et al.*, *J. Am. Chem. Soc.* **74,** *4321* (*1952*)). Differences in the types of carbon-carbon double bonds of the natural and the synthetic squalene were thus revealed. Natural squalene is a homogeneous compound containing only trisubstituted ethylene bonds. "Regenerated" squalene (squalene which has been passed through the solid hexachloride and is similar to the synthetic form) and synthetic squalene contain as much as 40 per cent of unsymmetrically disubstituted carbon-carbon bonds. A possible explanation of this difference is that the biosynthesis of cholesterol from squalene is a very stereospecific reaction, possible from natural squalene, but perhaps impossible from the regenerated or synthetic form.

To check this hypothesis, Tomkins, Dauben, Sheppard and Chaikoff (*loc. cit.*) fed rats for nine days a stock diet containing one or the other form of the hydrocarbon. The animals were then killed, their livers were rapidly excised and portions were incubated with carboxyl-labeled acetate. From 1.5 to 2.6 per cent of the C^{14} was recovered as cholesterol in the experiments with the rats fed only the stock diet. The feeding of a diet containing 3 per cent natural squalene resulted in a pronounced reduction in the recovery of the added acetate-C^{14} in cholesterol. The same dietary content of regenerated squalene had no such effect. The recoveries of $C^{14}O_2$ and fatty-acid C^{14} were unaffected by the ingestion of either type of squalene. The fact that the addition of natural squalene interferes with the incorporation of labeled acetate into cholesterol agrees with the hypothesis that the hydrocarbon dilutes a metabolic pool of a precursor of cholesterol. This theory is confirmed by the fact that the recoveries of $C^{14}O_2$ and fatty-acid C^{14} are unaffected. Squalene does not seem to act like a liver poison and it is unlikely that the decreased cholesterogenesis is a toxic effect. The fact that regenerated squalene shows none of these actions confirms the stereospecificity of the reaction. These experiments thus clear up an important point, hitherto confused by seemingly contradictory evidence. Squalene is probably an intermediary in the biosynthesis of cholesterol.

THE NATURE OF THE *LACTOBACILLUS BIFIDUS* FACTOR

The bacterial flora of the intestinal tract of breast-fed infants differs considerably from that of infants fed cow's milk. In the former, *Lactobacillus bifidus* predominates whereas a more mixed population exists in the gastrointestinal tract of infants fed cow's milk. In attempting to explain this difference in the bacterial flora of the intestinal system Paul György and his collaborators have studied the growth requirements of *L. bifidus*. As described in a previous review (*Nutrition Reviews* **12,** *24* (*1954*)), one interesting result of their investigations was the discovery of a variant of *L. bifidus* which required a growth substance present in human milk but almost absent from cow's

milk. György, R. F. Norris, and C. S. Rose, (*Arch. Biochem. Biophys.* **48,** *193* (*1954*)) have published additional information on this organism.

This strain, called *Lactobacillus bifidus* var. *Penn*, grows very poorly in a basal medium, but adequate growth was obtained following the addition of human breast milk. No morphologic differences were noted in this strain from other strains of *L. bifidus*, but it could be differentiated by serologic methods. With mutation it may lose its dependence on the human milk growth factor; however, it was not possible to adapt other strains of *L. bifidus* by growth in the presence of human milk so that the growth factor would be required. In a preliminary study of the properties of growth factor it was found not to be destroyed by autoclaving, but it was destroyed by ashing. Testing with known vitamins and microbiologic growth factors failed to reveal the identity of the substance present in human milk. It was found that cow's milk had only one fiftieth of the activity of human milk.

A more complete study of the natural occurrence of the growth factor for *L. bifidus* var. *Penn* has been published by P. György, R. Kuhn, C. S. Rose, and F. Zilliken (*Arch. Biochem. Biophys.* **48,** *202* (*1954*)). An assay system for the growth factor was devised. The activity of the substances tested was expressed in terms of units, defined as the growth-promoting effects of 0.06 ml. of a skimmed human milk preparation. The activities of human colostrum and milk were compared with those from a number of species. It was found that human colostrum contained the greatest amount of the growth factor, one unit being present in as little as 0.018 ml. Next in order of activity was rat colostrum, human milk, rat milk, and cow's colostrum. Very little growth factor was found in the milk of ruminants. The milks of the cat, monkey, dog, donkey, rabbit, mare and sow were intermediate in activity between cow's milk and human milk. In human milk the *L. bifidus* factor activity decreased with the duration of lactation. A number of other human secretions were tested. Meconium, semen, gastric juice, amniotic fluid, saliva and tears were all found to contain relatively large amounts of the factor. Because gastric mucin from hog was found to be active (*see Nutrition Reviews* **12,** *148* (*1954*)), a number of polysaccharides from *Pneumococci* types IV, V, VI, VIII, XVIII and XIX were tested, and were found active. The polysaccharides from several other types of pneumococci were found to be virtually inactive. Definite growth-promoting activity was present in ammonium salts, N-acetylglucosamine, and N-acetylgalactosamine, but in concentrations too high to explain the activity in milk and other natural secretions. It was suggested that these relatively simple substances might act as precursors of the growth factor.

The dialyzability of the growth factor for *L. bifidus* var. *Penn* has been studied (P. György, J. R. E. Hoover, R. Kuhn, and C. S. Rose, *Arch. Biochem. Biophys.* **48,** *209* (*1954*)). The growth activity of human milk could be separated into a dialyzable fraction, 40 to 75 per cent of the total activity, and a relatively nondialyzable fraction representing 25 to 60 per cent. The growth-promoting activity present in blood group substances, however, does not dialyze. From these physical measurements it appears that the factor needed for growth of *L. bifidus* var. *Penn* is not in a uniform state in natural secretions.

Further information concerning the nature of the *L. bifidus* var. *Penn* factor in milk is provided by A. Gauhe and co-workers (*Arch. Biochem. Biophys.* **48,** *214* (*1954*)). Considerable purification was achieved by removing fats by centrifugation, proteins by precipitation with barium and zinc ions, and salts by adsorption ion exchange on Amberlite. The removal of lactose from the preparations was achieved by adsorption on charcoal with subsequent elution, or by fractional precipitation. Hydrolysis of the

purified preparations yielded acetic acid, D-glucosamine, L-fucose, D-glucose, and D-galactose. The activity of the purified extracts was destroyed by acetylation, but could subsequently be restored by ammonolysis of the acetyl groups. Chromatography on charcoal and paper demonstrated at least four different compounds which had activity in the test system. All these compounds contained N-acetylglucosamine and galactose. Three of the compounds were levorotatory and contained fucose, the fourth was dextrorotatory and lacked fucose.

R. M. Tomarelli and co-workers (*Arch. Biochem. Biophys.* **48,** *225* (*1954*)) have been able to isolate a crystalline compound from hog gastric mucin which is active in *L. bifidus* assay. This starting material was selected after an assay of a number of substances had revealed that hog gastric mucin possessed the highest initial potency, 5 units per milligram. Following hydrolysis in 0.6 normal sulfuric acid for thirty minutes it was found that 35 per cent of the original activity was dialyzable. Purification was achieved by adsorption on charcoal and elution with 20 per cent acetic acid. After several chromatographic separations, fractions were obtained from which a crystalline, active compound was recovered. The active material was found to contain 12 units per milligram. Evidence is presented that this compound is a disaccharide, and is an acetylglucosamine rather than a galactoside.

These studies have provided much more definite information concerning the nature of the *L. bifidus* factor. At the present time a number of oligosaccharides containing amino sugars are known to sustain the growth of this organism. The general implications of these findings in terms of clinical pediatrics and general microbiology remain to be established.

GLUTATHIONE AND ASCORBIC ACID IN LIVER NECROSIS

The induction of liver necrosis by the feeding of diets deficient in sulfur-containing amino acids and in tocopherol has been demonstrated by H. P. Himsworth and his associates ("Lectures on the Liver and Its Diseases," *2d edition, Harvard University Press, Cambridge* (*1950*)) and by other investigators. The effect of such a necrogenic diet on the glutathione content of liver has been investigated by O. Lindan and E. Work (*Biochem. J.* **55,** *554* (*1953*)). This investigation took as its starting point the finding of G. Leaf and A. Neuberger (*Ibid.* **41,** *280* (*1947*)) that the feeding of a necrogenic diet led to a dramatic reduction in the glutathione content of liver.

The necrogenic diet employed by Lindan and Work consisted of 18 per cent dried baker's yeast, 73 per cent cornstarch, 5 per cent lard, 1 per cent cod liver oil and 3 per cent salt mixture. Each rat received in addition daily doses of thiamine, 20 micrograms; riboflavin, 25 micrograms; pyridoxine, 20 micrograms; and calcium pantothenate, 100 micrograms. Liver, blood and spleen were analyzed for glutathione, both oxidized and reduced, and for ascorbic acid. In addition, nitrogen, glycogen and water determinations were carried out on liver. The total glutathione concentration of the liver was markedly reduced during the first two weeks of feeding the necrogenic diet. At the same time, the amount of oxidized glutathione increased significantly in these prenecrotic livers. During this prenecrotic stage, the ascorbic acid content of the liver decreased slightly from that observed in suitable controls. Supplementation of the necrogenic diet with tocopherol had no detectable effect on the glutathione or ascorbic acid content of the livers. Tocopherol supplementation, however, did prevent the development of necrosis. The addition of cystine or methionine to the necrogenic diet protected to some

extent against the decrease in glutathione concentration of liver. In the case of methionine supplementation, the glutathione level was reduced by approximately 30 per cent, while the unsupplemented necrogenic diet led to a loss of nearly 75 per cent of the glutathione of the liver. No necrosis developed when the basal diet was supplemented with sulfur-containing amino acids. In this respect, then, tocopherol, cystine and methionine act alike. Livers taken still later and having fully developed necrotic changes showed a further drop in reduced glutathione but no change in the amount of oxidized glutathione present. These necrotic livers appeared to contain no ascorbic acid.

Prenecrotic livers showed less than normal concentrations of water and nitrogen, an increase in fat and essentially no change in glycogen concentration. Addition of tocopherol to the necrogenic diet had no effect on the histologic picture of prenecrotic livers. Supplementation with cystine and methionine, on the other hand, resulted in essentially normal appearance of the tissue. The addition of cystine alone resulted in essentially normal staining properties of the liver cytoplasm, but with increased fatty infiltration. The authors express some surprise at the fact that the antioxidant tocopherol had no effect on the liver levels of reduced glutathione and ascorbic acid, both of which are easily oxidized.

In a companion paper, O. Lindan and E. Work (*Biochem. J.* **55,** *562 (1953)*) studied glutathione and ascorbic acid changes in livers subjected to circulatory congestion caused by loose ligation at the base of the liver lobe. Congestion induced in this fashion causes necrosis which is, however, histologically different from that produced by the feeding of necrogenic diets. The nuclei remained largely normal, although some were pycnotic. The protoplasm took only a pale stain, but the liver cell chords were preserved, if somewhat compressed. In later stages, only a few nuclei remained visible, and there was marked infiltration of the liver parenchyma with polymorph-like cells. On the other hand, the necrogenic diets led to the development of livers in which the chords and sinusoids were not at all clearly defined. Circulatory congestion of the liver lobes led to sharp reduction in reduced glutathione and in ascorbic acid. The changes in nitrogen, water and glycogen were also similar to those observed in livers of animals maintained on the necrogenic diet.

MANNITOL AND SORBITOL AS DIETARY CONSTITUENTS

The availability of the sugar alcohols, mannitol and sorbitol, has stimulated interest in their nutritional use as substitutes for sugar in diabetic diets. The existing information on the metabolism of these substances has been summarized in a report of the Committee on Food and Nutrition of the American Diabetes Association, W. H. Olmsted, Chairman (*Diabetes* **2,** *132 (1953)*). Because the intestinal absorption is slow and several steps are required for the transformation to glucose, the ingestion of sorbitol does not raise the blood sugar to any great extent. In the diabetic individual the administration of sorbitol probably would increase the insulin requirement, but not to the same extent as glucose. The content of sorbitol in diabetic foods should be counted as available carbohydrate and the label of foods containing sorbitol should indicate the amounts present. Although small doses of sorbitol are well tolerated, amounts greater than 50 g. are laxative. In its use as a moisture conditioner of foods sorbitol is generally not incorporated in amounts which are nutritionally significant. When it is used as a sweetener it is only 60 per cent as sweet as glucose and probably will not

compete with the chemical sweeteners. A secure place for sorbitol in diabetic diets remains to be established.

In discussing mannitol the report states that "mannitol is absorbed so slowly and to such a slight degree that it does not affect the blood sugar or respiratory quotient of man." On the basis of reports following intravenous injection it is stated that "what is absorbed would be eliminated in the urine to the extent of 80 per cent or more." In experimental animals small amounts of mannitol have been converted to glycogen. Its use in diabetic diets is not promising because diarrhea appears after doses of 10 to 20 g.

The view that ingested mannitol is metabolized very poorly has been challenged by the studies of A. N. Wick, T. N. Morita, and L. Joseph (*Proc. Soc. Exp. Biol. Med.* **85,** *188 (1954)*), using C^{14}-labeled mannitol. Radioactive mannitol was prepared from uniformly labeled glucose by catalytic reduction. When mannitol was given to 2 rats by intraperitoneal injection 77 and 97 per cent of the radioactivity was recovered in the urine. During the twenty-four hours after injection 2.1 and 3.2 per cent of the administered C^{14} appeared in the expired carbon dioxide. The oral administration of mannitol gave different results. Under these circumstances between 46 and 63 per cent of the ingested C^{14} appeared in the expired air and from 8 to 13 per cent of the label appeared in the urine.

The difference in metabolism of mannitol when administered by mouth and by intraperitoneal injection could be attributed to either one of two possibilities. The mannitol could have been broken down by intestinal bacteria and the fragments absorbed from the gut and eventually oxidized and released as carbon dioxide in the expired air. On the other hand, the metabolism of mannitol might be largely carried out by the liver, and absorption from the gut through the portal system of the liver might provide a greater chance for metabolism prior to excretion in the urine. To distinguish between these possibilities 2 mg. of labeled mannitol was injected into the spleen. During the subsequent twenty-four hours 71 per cent of the administered C^{14} appeared in the expired carbon dioxide. On the basis of this experiment in a single animal it was concluded that intestinal bacteria are not essential for the extensive oxidation of mannitol in the body. It is known that the renal clearance of mannitol approximates the rate of glomerular filtration, and the authors postulate that following intraperitoneal injection the renal excretion is so prompt that little mannitol is available for metabolism.

SOLVENT TOXICITY RELATIONS WITH PROTEIN AND VITAMINS A AND E

The toxicities of carbon tetrachloride, pyridine and benzene for rats appear to involve the metabolism of vitamin E. This vitamin, under certain conditions, decreases the toxicity of these solvents, whereas under vitamin E-deficiency dietary conditions these solvents accentuate deficiency symptoms (E. L. Hove, *Arch. Biochem.* **17,** *467 (1948)*; *J. Nutrition* **50,** *361 (1953)*). In these investigations it was further observed that toxicities from these solvents could be alleviated partly by increased dietary casein levels.

Whether or not the ability of tri-o-cresyl phosphate (TCP) to induce acute symptoms of vitamin E deficiency, as demonstrated by H. Bloch (*Helvet. chim. acta* **25,** *793 (1942)*) and Bloch and A. Hottinger (*Z. f. Vitaminforsch.* **13,** *9 (1943)*) could be influenced by dietary protein, vitamin E, or vitamin A, was recently investigated by E. L. Hove (*J. Nutrition* **51,** *609 (1953)*).

The basal diet used contained methanol-extracted casein at 10, 20, or 60 per cent; salt mixture, 4 per cent; lard, 9 per cent; cod liver oil, 1 per cent; and sucrose to make the total 100 per cent. It was supplemented with the following (micrograms per gram of diet): thiamine, riboflavin, and pyridoxine, 5; calcium pantothenate, 30; niacin, 40; *i*-inositol, 200; 2 methyl-1,4-naphthoquinone, 2; and choline chloride, 2000.

Six weanling rats were used in each treatment. The treatments consisted of the three dietary levels of casein with and without 0.1 per cent TCP, and each of these with 0.1, 1.0, and 10 mg. of DL-alpha-tocopherol acetate per rat per day.

Vitamin E was partially protective against the growth inhibition induced by TCP and more so on low than on high protein diets. A combination of high tocopherol and high protein restored growth to nearly normal levels. Further evidence of protection was apparent from mortality data. All rats on the 10 per cent casein diet with no tocopherol died during the third or fourth week, whereas none receiving tocopherol on this casein level died.

In general, the tocopherol content of tissue fat was depressed by TCP feeding. Tissue tocopherol tended to be highest on the higher casein diets. Another change in tissue lipids as influenced by TCP was a consistent depression of arachidonic acid concentration (pronounced in carcass, somewhat less in brain, and least in liver). In all cases rats fed vitamin E along with TCP had normal arachidonic acid values, whereas vitamin E was without effect on arachidonic acid in lipids of control rats. The value for carcass fat indicated clearly that increases in dietary casein resulted in increased arachidonic acid concentration in carcass lipids.

The oleic acid concentration of liver lipids was depressed in vitamin E deficiency at all casein levels with or without TCP.

Linolenic acid was found only in fats of some of the TCP-fed rats without vitamin E and on low casein diets. Fatty livers were observed on the control 10 per cent casein diet supplemented with vitamin E, but were absent when the vitamin E supplement was omitted. This was observed in earlier work (E. L. Hove and J. O. Hardin, *Proc. Soc. Exp. Biol. Med.* **78,** *858 (1951)*).

Carotene utilization was reduced drastically (vitamin A less drastically) when 0.1 per cent of TCP was fed in a vitamin A-low diet. Such a diet supplemented with 3.7 mg. of beta-carotene per kilogram resulted in 0.63 per cent storage of vitamin A in liver, contrasted with 2.23 per cent storage in the absence of TCP.

The *in vitro* pro-oxidant effect of various compounds was also investigated. Pyridine, benzene, carbon tetrachloride, and TCP were excellent catalysts in the destruction of beta-carotene in a system containing methyl linolate hydroperoxide in absolute ethanol at 30°C. Pentachloronaphthalene was also found to be a catalyst in this system. The highly chlorinated naphthalenes have been implicated as the causative agents in bovine hyperkeratosis (R. W. Engel and W. B. Bell, *Nutrition Reviews* **11,** *97 (1953)*) and have been observed to reduce blood vitamin A values promptly when administered to cattle. Hove observed that in a limited number of bovine hyperkeratosis cases the blood carotene and tocopherol values were also depressed.

Each of the compounds studied causes a somewhat different pathologic picture in the animal, yet they show a common pattern in their mode of action.

These interesting results support the general thesis that the particular lipid-soluble toxic chemicals studied all act through a pro-oxidant effect on unsaturated lipids and fat-soluble vitamins. Variation in pathologic consequences of poisoning may simply imply differences in nutritional status of the subject with respect to the vulnerable nutrients.

THE INFLUENCE OF MAGNESIUM ON PROTEIN SYNTHESIS

It has become increasingly apparent that protein synthesis can not be considered as an isolated phenomenon. In order to achieve net protein synthesis more is needed than the proper mixture of amino acids. The importance of nonprotein calories and the time relation of nonprotein caloric intake to amino acid intake has been established. From the work of P. R. Cannon, L. E. Frazier, and R. H. Hughes (*Metabolism* **1,** *49 (1952)*; *Nutrition Reviews* **12,** *75 (1954)*) the requirement of inorganic cellular constituents for protein synthesis was suggested. These workers found that protein-depleted rats regained weight much faster if potassium was included in the repletion diet.

Next to potassium, magnesium is the most plentiful cation of the intracellular fluid. Evidence has been accumulated by W. Menaker that the availability of magnesium is essential for optimal protein synthesis under certain conditions. Menaker and I. S. Kleiner (*Proc. Soc. Exp. Biol. Med.* **81,** *377 (1952)*) subjected female rats to protein depletion by feeding a ration deficient only in protein for four weeks. The diet was similar to that used by Cannon *et al.* (*loc. cit.*) and contained only 2 per cent of the total calories as protein.

After the period of depletion the rats were divided into four groups. Group 1 received a basal diet containing 9 per cent of casein by weight, and a complete salt mixture. The food intake was limited to about the same amount as that ingested by the rats deficient in magnesium. During a ten-day period these rats gained an average of 30.5 g. Rats in group 2 received the same mineral supplement as group 1 but deficient in magnesium. These rats gained only 21 g. during the ten-day period. The mineral supplement of rats in group 3 contained only sodium and potassium chloride and was lacking in magnesium, calcium, phosphorus, iron and trace elements. On such a diet only 15.5 g. increase in body weight occurred in ten days. In the last group the diet contained no salt supplement and the weight gain was the poorest, 11.3 g.

In these experiments a gain in weight is assumed to be due to protein synthesis. To what extent this may be in error on diets of differing electrolyte content can only be a matter of speculation. It would appear that the restriction of magnesium intake produces almost as great a failure of protein synthesis, estimated in this way, as does a restriction in potassium intake. The authors state that no anatomic or physiologic sign of magnesium deficiency was observed other than the failure to gain weight.

D. V. Frost and H. R. Sandy (*Proc. Soc. Exp. Biol. Med.* **83,** *102 (1953)*) performed experiments to determine the effect of inorganic salts on the synthesis of protein. After a period of protein depletion rats were continued on a basal protein-deficient diet but were allowed 18.5 ml. daily of hydrolyzed fibrin fortified with tryptophan. This intake provided 120 mg. of nitrogen daily. Alterations in the salt mixture resulting in potassium-, phosphorus-, magnesium- and calcium-free diets were studied. During an eleven-week period the restriction of calcium and magnesium did not prevent rats from gaining weight at almost a normal rate. Phosphorus restriction greatly inhibited weight gain and potassium deficiency was associated with weight loss and death in several of the experimental animals.

The explanation for the conflicting results on the effect of magnesium deficiency on protein synthesis has been sought by W. Menaker (*Proc. Soc. Exp. Biol. Med.* **85,** *149 (1954)*). This author noted that the weight gain in the experiments of Frost and Sandy was as small as that which occurred in the magnesium-deficient animals in his earlier experiments, presumably due to the low nitrogen intake. For this reason the

effect of nitrogen intake on magnesium requirement for protein synthesis was studied using the same experimental design described in his earlier paper. The gain in weight of protein-depleted rats when fed diets containing 7 and 14 g. of protein with and without magnesium in the salt mixture was measured.

At the lower protein intake, providing 120 mg. of nitrogen daily, rats gained 14.3 g. during ten days with a complete mineral supplement. When magnesium was lacking the weight gain was still 12.3 g. A strikingly different result was obtained at the higher protein intake, 14 per cent casein. Rats fed this diet plus a complete mineral supplement gained 36 g. during ten days. When magnesium was withheld the mean weight gain was 5.8 g., a highly significant difference. It is thus apparent that the gain in weight on a magnesium-deficient ration is almost twice as great with a low protein intake as compared to a moderate protein intake. Some of the failure to gain weight can be attributed to anorexia which appeared on the fourth day. A marked vasodilatation of the skin of the ears appeared on the sixth day, a recognized manifestation of magnesium deficiency. These findings suggest that the magnesium requirement increases with increased protein intake. Moreover, the increased magnesium is not required solely for inclusion in the tissue formed. The full explanation for the increased need for magnesium remains to be determined.

STUDIES ON NITROGEN BALANCE

Studies on the effects of amino acid deficiency on nitrogen balance are often complicated by the voluntarily reduced food intake of the deficient animal. The method of paired feeding does not altogether circumvent this difficulty, since the deficient animals and their restricted controls will be reduced to levels of nitrogen and caloric intake which will in themselves adversely affect nitrogen balance. In a study of the effects of methionine and histidine deficiencies on nitrogen balance in young rats, R. M. Forbes and L. Vaughan (*J. Nutrition* **52,** *25 (1954)*) have resorted to the forced feeding of experimental rations in order to maintain nitrogen and calorie intakes at adequate levels.

In the first experiment rats weighing between 60 and 80 g. were limited to a daily intake of 6 g. of stock ration for a period of two weeks. Subsequently the animals were force-fed a complete synthetic ration for a period of six days. This was followed by an eight-day period during which some animals were force-fed a methionine-free ration, while some were continued on the complete diet. For another five-day period all animals received the complete ration. The daily collections of urine were analyzed for nitrogen.

Early striking effects of amino acid deficiency were seen in these experiments. While the animals force-fed the complete ration gained between 1.5 and 2 g. of body weight per day, the animals receiving the methionine-deficient diet barely maintained their original body weights and in some instances actually lost weight although receiving the same quantity of food as the controls on complete rations. Of the 5 animals maintained on the methionine-deficient diet 2 died on the eighth day of the deficient regimen. Deficiency symptoms included the appearance of a porphyrin-like pigment around the nose and forepaws, followed by hyperemia and hair loss about the throat and paws. The animals became lethargic and presented increasing difficulty in feeding. They appeared bloated, due, as determined at autopsy, to the retention of food in the stomach and intestine. The livers were extremely fatty.

The authors speculate that the forced feeding of large amounts of an unbalanced

amino acid mixture might account for the early onset of deficiency symptoms. Strikingly rapid recovery from the deficiency state was observed on refeeding of the complete ration. Animals force-fed the complete ration excreted in the urine 64 per cent of the nitrogen fed. On the other hand, animals deficient in methionine excreted 92 per cent of the administered nitrogen. These results are in complete harmony with the observations on body weight changes in that the animals receiving the complete diet gained between 1.5 and 2 g. per day, while the methionine-deficient rats barely maintained their original body weights.

Since the authors felt that the eight-day exposure to the deficient ration used in the first experiment was too long, a second experiment was devised. Here the animals were fed a commercial stock ration for one week, being restricted to 6 g. per day during the last three days of the experimental period. Thereafter, they were fed by stomach tube a synthetic ration containing the individual amino acids in place of protein. The quantities of diet administered were 4.5 g. per day during the first two days and 6 g. per day for a period of three days. Thereafter followed a six-day period during which half the animals received the synthetic diet minus histidine while the remaining half received the same diet minus methionine. After six days on the deficient regimens, both groups were returned to the complete ration for a six-day recovery period.

In this experiment the histidine-deficient animals lost 3.2 g. of body weight during the six-day deficiency period. The methionine-deficient animals lost 6.0 g. A substantial number of the deficient animals died within twenty-four hours after being returned to the complete ration. As in the first experiment, nitrogen balance was strongly positive when the complete ration was administered to these young, growing animals. During the six days on the amino acid-deficient diet, nitrogen balance dropped nearly to zero, becoming again strongly positive during the six-day recovery period.

The third experiment was designed to test the effect of depletion of protein stores on nitrogen balance during the feeding of methionine-deficient rations. Young rats were maintained for seven days on a nitrogen-free diet. Thereafter one group was continued on the methionine-free ration, another group received a ration containing 0.15 per cent methionine, while a third group received 0.6 per cent methionine. During the week on a nitrogen-free ration, the animals lost 17 per cent of their body weight. Within two days after the beginning of forced feeding, 50 per cent of the experimental animals died, the majority exhibiting severe retention of food in the stomachs and intestines.

M. W. Marshall and M. Womack (*J. Nutrition* **52,** *51 (1954)*) have extended an earlier report on the relative effects of sucrose and dextrin on nitrogen balance when low levels of amino acids are fed. When essential and nonessential amino acids were fed to adult rats at the 0.1 per cent level, a daily nitrogen balance of −23.8 mg. was observed with sucrose as the dietary carbohydrate while with dextrin the balance was −15.5 mg. The addition of 1.65 per cent glutamic acid to furnish additional nitrogen brought about a significant improvement in nitrogen balance with both carbohydrates. Dextrin was, however, still superior to sucrose under these conditions. Raising the glutamic acid level of the sucrose-containing diet to 6.45 per cent resulted in poorer nitrogen balance than when only 1.65 per cent glutamic acid was employed. This was not the case for the dextrin diet. When the essential amino acids were fed at a 0.2 per cent level and the nonessential ones at a 0.1 per cent level, nitrogen equilibrium was achieved. Animals maintained on nitrogen-free diets for eighteen days prior to testing showed more or less the same results except that nitrogen balance tended to be more favorable. The feeding of glutamic acid at the 6.45 per cent level in the

sucrose diet did not have the adverse effect noted in animals not previously depleted of protein.

When the essential amino acids were fed at the 0.1 per cent level, the use of sucrose as dietary carbohydrate resulted in larger than normal livers. These enlarged livers tended to have a higher percentage of fat and a lower concentration of water and protein. As expected, the animals previously depleted of protein possessed less liver protein than the nondepleted rats.

Using dogs, man and rats for nitrogen balance studies, H. N. Munro and T. W. Wikramanayake (*J. Nutrition* **52,** *99 (1954)*) investigated the time factor in the relationship between intake of carbohydrate and fat protein metabolism. In rats and dogs the protein-sparing action of carbohydrate could be demonstrated even if the protein and carbohydrate were administered twelve hours apart. Fat was found to have a similar favorable effect on nitrogen balance. The fat supplement, like carbohydrate, did not need to be administered with the protein.

Earlier experiments had shown a favorable effect of carbohydrate (but not of fat) when administered simultaneously with the protein, an effect which was not demonstrable if a significant time interval elapsed between the feeding of the two nutrients. The authors conclude that carbohydrate affects nitrogen balance in two ways: (1) a direct influence on protein utilization depending on the simultaneous feeding of protein and carbohydrate and specific for carbohydrate, and (2) an influence on "endogenous" metabolism independent of the time of administration and shared by carbohydrates and fats. It is essentially the second effect which is supported by the data reported in this paper.

NUTRITION EXPERIMENTS WITH ARMY RATIONS

In an attempt to maintain variety as well as nutritional adequacy, military rations for use under combat conditions have received considerable research attention in recent years. Such rations must meet the requirements of compactness and of stability under prolonged storage in a variety of adverse conditions. Furthermore, insofar as is possible, attempts have been made to insure that such rations will be nutritionally adequate for the soldier. In the past, the vitamins around which interest has centered are vitamin A, ascorbic acid, thiamine, riboflavin and niacin.

The 5-in-1 type of combat ration ("Operational Rations, Ration Supplements and Food Packets," *Food and Container Institute, Inc., Associates, Chicago, Illinois (1952)*) is an example of a ration which accomplishes compactness and yet meets the requirement of acceptability since it consists of about 45 different processed food items.

The nutritional adequacy of the 5-in-1 ration for monkeys was recently tested by workers at the University of Wisconsin (D. V. Tappan and C. A. Elvehjem, *J. Nutrition* **51,** *469 (1953)*) in cooperation with the Quartermaster Food and Container Institute for the Armed Forces. Work with rats indicated that the 5-in-1 ration supported satisfactory rates of growth in this species (Tappan, U. J. Lewis, A. H. Methfessel, and Elvehjem, *Ibid.* **51,** *479 (1953)*). Work with the monkey was of interest both because of the closer relationship to man and because this species can be used for evaluating the ability of a ration to sustain good nutritional health in adult animals for a period of several years.

Six monkeys were used in the experiment and were maintained on the 5-in-1 ration for periods of sixteen to twenty-six months. Two of the monkeys were fed the ration without the soluble coffee product (ascorbic acid-supplemented), since it was observed

that animals demonstrated a dislike for the rations containing this product. A general weakness, loss of interest in food, and swelling around the eyes which developed under this ration in four months was corrected by supplementation with ascorbic acid. After one month of ascorbic acid supplementation, a secondary deficiency began which was represented by a declining body weight for about four months. This was reversed by a combined supplementation of pyridoxine, folacin, ascorbic acid and inositol; the former three proved, by further studies, to be the important ones in this supplemental mixture. Supplementing the 5-in-1 ration with folacin and ascorbic acid only resulted in a decline in blood hemoglobin to a low of 5 g. per 100 ml. of blood. Although supplements of pyridoxine, calcium pantothenate, thiamine and vitamin B_{12} (10 micrograms per day) were tried, the hemoglobin did not rise above 8.0 g. per cent during the remaining thirteen months of the experiment. Early work had indicated that (next to folacin and pyridoxine) pantothenate, thiamine and vitamin B_{12} were the most limiting vitamins in the ration (L. M. Scott, L. A. Maynard, and L. C. Norris, "Report to Quartermaster Food and Container Institute on the Nutritive Value of Army Rations as Determined by Growing Chicks" (*1951*)).

The importance of pyridoxine supplementation was demonstrated by the Wisconsin workers in 3 of the monkeys in the study. This vitamin seemed to be capable of maintaining more nearly normal hemoglobin levels when used as a supplement in addition to folacin and ascorbic acid. These results support the earlier observation of the Wisconsin group that a deficiency of pyridoxine eventually occurs in experimental animals when fed highly processed rations (E. M. Sporn and C. A. Elvehjem, *J. Nutrition* **35,** *549* (*1948*); U. D. Register, U. J. Lewis, W. R. Ruegamer, and Elvehjem, *Ibid.* **40,** *281* (*1950*)).

It was observed throughout these studies that injection of antibiotics to assist in controlling secondary complications during times of deficiency proved to be a useful innovation in nutrition studies with monkeys.

The high vitamin B_6 requirement of animals receiving highly processed rations was further investigated by using the combat ration, type K. This ration is a more compact ration than the 5-in-1. It is also generally less complete nutritionally, less acceptable, and is at present obsolete. However, further studies aimed at improving a ration of this type were indicated for reasons of ease of manufacture, storage and shipment.

In studies with rats, it was learned that the ham and egg and the pork units of the type K ration were in part responsible for the increased pyridoxine requirement of weanling rats. No such effect could be demonstrated, however, when these items of the ration were fed apart from the remaining portions of the ration. It was possible to effect marked improvements in the K ration by substitution of other meat units for the pork unit. For example, substitution of canned beef for the pork and egg mixture resulted in significant growth response in rats. However, supplementation of the beef ration with pyridoxine produced a further marked improvement in growth. Other protein portions of the K ration (*i.e.*, egg, cheese, or other meats), however, did not significantly alter the response to pyridoxine.

The unusual vitamin requirements which result from the use of highly processed rations is a very interesting observation and deserves considerably more study. Studies of this nature should also lead to the development of compact rations of improved nutritional quality. Famines or other emergencies, including military combat operations, always present the need for stable, compact and nutritionally acceptable rations.

BIOCHEMICAL EFFECTS OF VITAMIN B_6 DEFICIENCY

Vitamin B_6 has been shown to be concerned in protein metabolism. Pyridoxal phosphate serves as a coenzyme for certain amino acid decarboxylases and transaminases (*see Nutrition Reviews* **4,** *232 (1946)*), and the requirement for vitamin B_6 is increased when rats are given increased quantities of dietary protein (E. C. Miller and C. A. Baumann, *J. Biol. Chem.* **157,** *551 (1945)*). Vitamin B_6 has also been implicated in fat metabolism. A. G. Hogan and L. R. Richardson (*J. Nutrition* **8,** *385 (1934)*) fed rats a purified diet containing ultraviolet-irradiated yeast as the vitamin source and reported the development of a dermatitis which resembled that described by G. O. Burr and M. M. Burr (*J. Biol. Chem.* **82,** *345 (1929)*) in rats fed a diet deficient in essential fatty acids. It was later shown that the antidermatitis factor which was destroyed by ultraviolet irradiation was vitamin B_6. Later T. W. Birch (*Ibid.* **124,** *775 (1938)*) reported that both vitamin B_6 and essential fatty acids were necessary to prevent the dermatitis and suggested that vitamin B_6 was required for the utilization of the essential fatty acids.

A comprehensive study of the biochemical changes which accompany vitamin B_6 deficiency in the rat has been reported by J. R. Beaton and co-workers (*J. Biol. Chem.* **207,** *385 (1954)*). The data are particularly valuable because they were obtained at various time intervals after the animals were placed on the deficient diet and thus indicate the relative contributions of the various biochemical changes to the general condition of the animal. Rats were given a purified basal diet containing 20 per cent casein, 20 per cent corn oil, and the usual vitamins except vitamin B_6. For a preliminary seven-day period the animals were each given 50 micrograms of pyridoxine hydrochloride daily. They were then divided into three groups; one group was given the basal vitamin B_6-free diet, the other two groups were given this diet plus 50 micrograms of pyridoxine hydrochloride per rat per day. Animals in one of the groups receiving pyridoxine were pair-fed with the deficient animals, rats in the third group being given food ad libitum. The experimental period lasted eight weeks. Animals were killed at various intervals during this period for the determination of various body constituents and tissue enzymes.

The rats receiving the vitamin B_6-deficient diet grew at a reduced rate from the onset of the experiment. Also, liver and carcass concentrations of vitamin B_6 were reduced after only one week on the deficient diet. It would appear that although the animals were given pyridoxine for a week prior to the feeding of the deficient diet they were unable to store appreciable quantities of the vitamin. In this connection it has been reported that monkeys, previously fed adequate pyridoxine, begin to lose weight within two weeks after the vitamin is withdrawn from the diet (G. A. Emerson, G. E. Boxer, and E. W. Gilfillan, *Fed. Proc.* **13,** *456 (1954)*). There was no marked effect of vitamin B_6 deficiency on the activity of liver aspartic-glutamic transaminase; however, liver alanine-glutamic transaminase was influenced by the deficiency. The activity of this latter enzyme was found to increase steadily throughout the eight-week experimental period when the rats were given vitamin B_6. In contrast, there was no increase in the activity of the enzyme in livers of vitamin B_6-deficient rats. After six weeks of feeding, the vitamin B_6-deficient rats exhibited approximately half the activity of this transaminase that was found in livers from vitamin B_6-fed animals.

The rate of urea formation by liver slices was increased when the rats were deficient in vitamin B_6. This observation is in agreement with the earlier report by Beaton and

co-workers (J. R. Beaton, F. I. Smith, and E. W. McHenry, *J. Biol. Chem.* **201,** *587 (1953)*), in which it was shown that vitamin B_6 deficiency resulted in elevated blood urea levels.

In all the enzyme measurements similar results were obtained with the pair-fed pyridoxine-supplemented animals and those given food ad libitum.

Perhaps the most interesting data reported concerned the net gains in various carcass constituents by rats fed these diets. Total carcass moisture, protein, and total crude fatty acids were determined on rats killed at the start of the experiment and on rats fed the experimental diets for the eight-week period. The net gain per rat in the various constituents was then calculated. The vitamin B_6-ad libitum-fed rats gained considerably more carcass water than either of the other two groups. The vitamin B_6-pair-fed rats gained only slightly more water than did the vitamin B_6-deficient animals. The vitamin B_6-deficient rats and the vitamin B_6-pair-fed rats gained the same amount of protein which was only slightly less than the amount gained by the rats fed ad libitum.

The most striking differences were encountered in the gains of total crude fatty acids. The vitamin B_6-fed animals, either pair- or ad libitum-fed, gained approximately ten times the quantity of fatty acids gained by the deficient animals. The difference in body weight gain between the vitamin B_6-deficient rats and the vitamin B_6-pair-fed rats was then due chiefly to the difference in gain of body fat.

The results of these experiments indicate that fat metabolism is seriously deranged in the vitamin B_6-deficient rat. Since the basal diet contained adequate quantities of fat and essential fatty acids, the inability of the vitamin B_6-deficient rats to deposit carcass fat cannot be readily ascribed to an impairment in fatty acid synthesis. It would appear that vitamin B_6 is somehow necessary for the deposition of fatty acids in tissue fat. This recalls the earlier suggestion of Birch (*loc. cit.*) that vitamin B_6 may be necessary for the utilization of the essential fatty acids.

FRUCTOSE METABOLISM IN THE LIVER

In a recent review on this subject (*Nutrition Reviews* **12,** *154 (1954)*) two metabolic pathways for liver fructose-1-phosphate were discussed. In particular, the work of E. Helmreich, S. Goldschmidt, W. Lamprecht, and F. Ritzl (*Z. f. physiol. Chem.* **292,** *184 (1953)*) on the catabolism of fructose-1-phosphate was reviewed, and that of G. T. Cori, S. Ochoa, M. W. Slein, and C. F. Cori (*Biochim. et biophys. acta* **7,** *304 (1951)*) on the "assimilative" pathway of fructose-1-phosphate was discussed.

Recent work carried out at the University of Louvain, Belgium, by H. G. Hers and T. Kusaka (*Biochim. et biophys. acta* **11,** *427 (1953)*) has added information concerning the metabolism of fructose-1-phosphate in the liver. Using extracts prepared from perfused rat, rabbit and guinea pig livers, they found that fructose-1-phosphate in the presence of aldolase and magnesium ions is broken down rapidly into the trioses, glyceraldehyde and dihydroxyacetone phosphate (phosphodioxyacetone). The latter compound may be partially isomerized to glyceraldehyde phosphate through the enzymatic action of triosephosphate isomerase, and the two triose phosphates may then recondense under the catalytic influence of aldolase to form fructose-1,6-diphosphate.

This latter compound can be hydrolyzed by fructose-1,6-diphosphatase to fructose-6-phosphate. Fructose-1,6-diphosphatase has an absolute requirement for magnesium ions. In the absence of this cation very little fructose-1-phosphate is catabolized

since the equilibrium is greatly in favor of fructose-1-phosphate synthesis. In the presence of magnesium ions fructose-1,6-diphosphate disappears, shifting the equilibrium in favor of the breakdown of more fructose-1-phosphate. The over-all scheme for the breakdown of the latter substance and reconstitution to form fructose-6-phosphate may be represented according to Hers and Kusaka as follows: fructose + ATP → fructose-1-phosphate ⇄ glyceraldehyde + dihydroxyacetone phosphate; glyceraldehyde phosphate + dihydroxyacetone phosphate ⇄ fructose-1,6-diphosphate + Mg^{++} → fructose-6-phosphate ⇄ glucose-6-phosphate.

A new enzyme, triokinase, was partially purified by Hers and Kusaka. In the presence of ATP it phosphorylates glyceraldehyde and dihydroxyacetone at approximately the same rate.

This pathway for the conversion of fructose-1-phosphate to fructose-6-phosphate differs from that hypothesized by Cori *et al.* (*loc. cit.*). These authors considered that fructose-1-phosphate was converted into fructose-6-phosphate via an unknown intermediate by means of the enzymatic action of a phosphofructomutase. They based their argument on the formation of inorganic phosphate and reducing sugars which they assumed were hydrolysis products of the hexose-6-phosphate formed at the expense of fructose-1-phosphate. Hers and Kusaka could find no evidence for such a conversion. Their liver preparations (pH5) were devoid of any hydrolytic effect on either glucose-6-phosphate or fructose-6-phosphate maintained in equilibrium by means of phosphohexoisomerase. They found that the reducing sugar formed at the expense of fructose-1-phosphate was not a hexose but a triose, probably glyceraldehyde. Approximately one mole of this substance was formed per mole of fructose-1-phosphate disappearing. These facts led Hers and Kusaka to reject the one-step conversion of fructose-1-phosphate to fructose-6-phosphate hypothesis proposed by Cori *et al.* In place of this they postulated the scheme presented above.

Hers and Kusaka also were unable to find any evidence in support of the claims of F. E. Leuthardt, E. Testa, and H. P. Wolf (*Tagg. d. Schweiz. Physiol. u. Pharm. Genf, 25 (1952)*; *Helvet. chim. acta* **36,** *227 (1953)*) of the existence of two different aldolases: (1) a 1-phosphofructaldolase catalyzing the reversible reaction between fructose-1-phosphate, glyceraldehyde and dihydroxyacetone phosphate; and (2) a "1,6-diphosphofructaldolase" catalyzing the reversible reaction between fructose-1,6-diphosphate and triose phosphate. They found that liver aldolase saturated with substrate splits fructose-1-phosphate and fructose-1,6-diphosphate at exactly the same speed. The latter substrate, however, possesses one hundred times more affinity for aldolase than the former. Part of the differences observed may also be attributed to the use of muscle aldolase by Leuthardt *et al.* which has different properties from the liver enzyme.

From the above discussion the most recent evidence indicates that both the catabolic and anabolic pathways for fructose-1-phosphate are the same until the triose (C_3) stage is reached. At the C_3 level the trioses glyceraldehyde and dihydroxyacetone phosphate formed from fructose-1-phosphate may be acted upon by the enzymes triokinase and triosephosphate isomerase forming glyceraldehyde phosphate. This latter compound may be further catabolized to "active acetate" (acetyl CoA) via pyruvate, or alternatively recondense with a molecule of dihydroxyacetone phosphate to form fructose-1,6-diphosphate. The latter compound may then be converted to glucose-6-phosphate via fructose-6-phosphate. Glucose-6-phosphate represents the first common intermediary in fructose and glucose ana-

bolism to glycogen. It is interesting to note that in the liver one mole of ATP and a single enzyme (glucokinase) suffice to convert glucose into glucose-6-phosphate while six enzymatic steps and two moles of ATP are required to arrive at the same compound starting with fructose. In spite of this, fructose is more rapidly transformed into liver glycogen than glucose, indicating that the glucokinase (hexokinase) reaction is the rate-limiting step for the conversion of glucose into glycogen.

HYDROCEPHALUS ASSOCIATED WITH DEFICIENCY OF VITAMIN A

Disturbances of various neurologic functions in vitamin A-deficient animals and patients have long been recognized. However, there is still some question whether there are primary and specific central nervous system lesions associated with vitamin A deficiency. Thus, there is considerable evidence to indicate that the pathologic changes are secondary to mechanical injury resulting from disproportionate growth of the skeleton and nervous systems (E. Mellanby, *J. Physiol.* **94,** *380* (*1938*); *Nutrition Reviews* **10,** *343* (*1953*)). In this regard, S. B. Wolbach and O. A. Bessey (*Arch. Path.* **32,** *689* (*1941*); *Nutrition Reviews* **7,** *319* (*1949*)) have emphasized the irregularity in the intensity and distribution of the nervous system lesions in vitamin A-deficient rats. Contrariwise, J. W. Millen, D. H. M. Woollam, and G. E. Lamming (*Lancet* **II,** *1234* (*1953*)) have recently presented evidence to suggest a primary central nervous system lesion in vitamin A-deficient rabbits.

Five does received a vitamin A-deficient diet for fourteen weeks before mating with normal males. Three of the does were of an inbred strain of stock rabbits to which their mates also belonged. The fourth doe was of the same strain, but of a line which had been separated out for color some years previously. The fifth doe was of another breed and was mated to an outbred male.

The offspring of these animals were continued on a vitamin A-deficient diet after weaning. Controlled does received the same basic diet plus an ample supplement of vitamin A. No details of the basic vitamin A-deficient diet are given. All five vitamin A-deficient does gave birth to young. All 13 offspring of 3 of the rabbits showed signs of lesions of the nervous system (particularly paralysis of the hind limbs), as did 3 in the other two litters. The remaining young (number not stated) in the latter two litters had not shown any signs of central nervous system disturbance at the time of publication of the report. Roentgenologic examination of the skull of all the young rabbits with neurologic disorders revealed enlargement and a "beaten silver" appearance of the calvaria, suggesting hydrocephalus. The diagnosis was established when gross dilatation of the lateral and third ventricles and thinning of the surrounding cerebral cortex and white matter was found at autopsy. The optic nerves were constricted at the optic foramina. Herniation of the cerebellum through the foramen magnum occurred in one rabbit. Further examination revealed what the authors consider the probable reason for the dilatation of the lateral and third ventricles—a marked stenosis of the cerebral aqueduct at the level of the superior colliculus. Above this level, the lumen of the aqueduct was dilated; distal to this level, the lumen was normal. Colloidal carbon injected into the lateral ventricles did not pass the point of constriction in the aqueduct. No details of the microscopic findings of this area are given by the authors. The fourth ventricle in all rabbits was normal and no deformities of the foramen magnum

or base of the skull were observed. There was no evidence of meningitis. No mention is made of the rate of growth of the bones of the calvaria in these animals as compared with the controls.

Fourteen of the 16 vitamin A-deficient offspring showing the neurologic changes were of a strain which had been inbred for over twenty-five years. No instances of spontaneous congenital hydrocephalus had been noted among this breed during these years, nor were any abnormal functional or anatomic changes observed in the control rabbits receiving vitamin A in the current study.

Among human beings, hydrocephalus is considered to be congenital in origin in most instances. Among the lesions concerned with hydrocephalus is abnormality (stenosis, forking, and septum formation) of the cerebral aqueduct (D. S. Russell, *Med. Res. Council, London, Special Report Series No. 265 (1949)*).

Abnormality of the aqueduct has been calculated as occurring about once in every 9000 births. Whether vitamin A deficiency may play a role in the pathogenesis of human hydrocephalus merits investigation. In this regard, D. Cornfeld and R. E. Cooke (*Pediatrics* **10,** *33 (1952)*; *Nutrition Reviews* **10,** *327 (1952)*) have reported facial palsy and hydrocephalus developing in a young infant fed a vitamin A-deficient diet for three months starting at the age of 2½ months. Following treatment with vitamin A, the facial palsy and hydrocephalus gradually disappeared, and three and one-half months after the start of vitamin A therapy there was no evidence of physical or mental retardation. Cornfeld and Cooke attributed the hydrocephalus to failure of growth of the skull, with consequent compression of the normally growing central nervous system and interference with the absorption of the spinal fluid. In support of this hypothesis is the rapid head growth which occurred during the vitamin A therapy. This interpretation of the sequence of events in this child is consistent with the observations of Mellanby, Wolbach and others in vitamin A-deficient animals. On the other hand, Millen, Woollam, and Lamming have interpreted their findings in rabbits as indicating that the hydrocephalus observed in the offspring of the vitamin A-deficient animals was directly consequent to stenosis of the cerebral aqueduct. They feel that this lesion is a primary pathologic change in vitamin A-deficiency. No explanation for the development of the stenosis of the aqueduct is offered.

It is to be hoped that in future reports the authors will include information as to the composition of the basic diet and quantities consumed, and detailed descriptions of the gross and microscopic pathology of the central nervous system and of the bones, particularly of the calvaria. Similar studies should be carried out in other breeds of rabbits and in other species. Various basic diets should be used and, among the controls, pair-fed animals should be included.

NOTES

Letters to the Editor

Dear Sir:

May I call to your attention an error in the review "Carotene Utilization" in the February 1954 issue of *Nutrition Reviews* (**12,** *59 (1954)*), page 60, column 1, paragraph 3, line 7, in which it is stated that, "It (blood vitamin A value) then dropped in one week to about 32 micrograms per cent and remained at this level throughout the test period." A review of the original article shows that the blood vitamin A value dropped from 45 to 32 micrograms per cent during the following month.

It might be mentioned that the blood vitamin A levels associated with casein or lactalbumin approached, and occasionally attained, values which were significantly (P = 0.05) higher than those associated with gluten or zein-casein.

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Dear Sir:

I appreciate the review with favorable comments on the possible human applications of my recent work on "Improvement of Corn Proteins with Amino Acids," which appeared in the March issue of *Nutrition Reviews* (**12,** *70 (1954)*) and which was apparently written by one of your associate editors. I would like to point out several errors which appeared in the text of the first paragraph. Pecora and Hundley reported on the improvement of the proteins in polished rice with threonine and lysine but not in the presence of lysine and tryptophan. Also, I obtained an appreciable response by supplementing the proteins in patent wheat flour with threonine in the presence of lysine, but a much more marked response was secured by addition of threonine in the presence of lysine and valine, but certainly not in the presence of lysine and tryptophan. The reference to this work is not *Arch. Biochem. Biophys.* **39,** *463 (1952)* but *J. Nutrition* **50,** *235 (1953)*.

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Composition of Yeast Protein

Dried yeast has long been recognized as an excellent source of the B-vitamins, but as a main source of protein it is inadequate, partly due to its low content of methionine and cystine. Only up to one half of the protein in the ration of chicks or rats can be replaced by yeast protein without decreasing growth rates. Several commercial yeasts and 20 species of laboratory yeasts have been studied in regard to their contents of methionine and cystine by J. S. Chiao and W. H. Peterson (*J. Agr. Food. Chem.* **1,** *1005 (1953)*).

Methionine contents of 7 commercial yeasts varied from 0.48 to 0.75 per cent. Similar values of the 20 species studied ranged from 0.17 to 1.0 per cent when grown on a Hawaiian molasses medium. Addition of ammonium salts to a beet-molasses medium increased the yield of yeast and the nitrogen content, but methionine remained constant. Presumably up to a certain limit the added nitrogen is converted to protein of fixed methionine content. Magnesium salts further increased yield but not methionine content.

Several species were studied in more detail. Methionine content was not increased by addition of cystine, threonine, and choline; however, cystine greatly increased the yield. Addition of methionine to synthetic mediums did not increase the methionine content of the yeast.

These feeding experiments on yeast do not appear promising so far as improvement of quality or composition of yeast protein are concerned. Differences between species indicate the importance of genetic factors.

Changes in Peanut Composition During Roasting

Changes in the composition of peanuts during processing have been discussed in detail by C. L. Hoffpauir (*J. Agr. Food Chem.* **1,** *668 (1953)*). Since the peanut kernel contains approximately 50 per cent oil and 30 per cent protein, these substances are of particular interest. A content of from 0.03 to 0.05 per cent alpha-tocopherol and gamma-tocopherol in peanut oil contributes much to its excellent stability toward oxidation. Two globulins, arachin and conarachin, have been identified, but neither has been purified completely. Electrophoretic analysis has revealed two major and two minor components in peanut protein. Residual protein after removal of arachin and conarachin contains 2.9 per cent sulfur, which is very high for a vegetable protein.

Any consideration of roasting must recognize effects due to interaction between various constituents as well as thermal decomposition and volatilization. Peanuts with an initial moisture content of 4 to 6 per cent are roasted at an internal temperature of 265 to 300°F., reducing the moisture to 1 per cent. A large part of the thiamine is destroyed, while niacin, choline and riboflavin are but little reduced. Proteins are denatured, as shown by solubility changes, but their amino acid contents and nutritive value are not much altered. Free fatty acid content of the oil does not change. Apparent total sugars decrease, but starch content is not changed. Volatile materials lost during roasting include furfural derivatives, vanillin, ammonia, hydrogen sulfide, and diacetyl. Similar substances are expelled during roasting of coffee. Sugar probably caramelizes to some extent and is further reduced by reaction with amino groups of proteins and amino acids to produce non-enzymatic browning. These reactions, complex in nature, produce characteristic flavors and aromas. Extensive browning usually causes a loss of nutritive value of the protein. Sulfur loss indicates some thermal decomposition of proteins. Enzymes are inactivated. The oil undergoes no great change, but flows freely throughout the kernel and wets the entire cellular structure.

Ferric Versus Ferrous Iron for Prevention of Hypochromic Anemia in Infants

Unless iron is added to the diet of infants hypochromic anemia frequently develops. The relative effectiveness of ferric and ferrous iron in preventing this anemia is of considerable interest to the pediatrician. W. L. Niccum, R. L. Jackson, and G. Stearns (*Am. J. Dis. Child.* **86,** *553 (1953)*) have reported the results of experiments in which full term infants were treated with ferric or ferrous iron.

The infants were in the metabolism ward of a hospital and were given modified whole milk feedings. Growth and development were reported to be satisfactory. Beginning at the age of 3 months 115 infants were given supplements of ferric ammonium citrate and 37 comparable infants were given ferrous sulfate. For both regimens the initial dosage was 5 mg. of iron daily, which was increased to 10 mg. at 6 months of age. Observations were made until the infants were 12 months of age.

The average hemoglobin level obtained between the twenty-eighth and thirtieth weeks for infants receiving ferrous iron was 12.7 g. per cent (standard error 0.26) while the value for infants receiving ferric iron was 11.8 g. per cent (standard error 0.19). At an age of from 37 to 39 weeks infants receiving ferrous iron exhibited an average hemoglobin level of 13.1 g. per cent (standard error 0.31) as compared to 11.7 g. per cent (standard error 0.20) for the group receiving ferric iron. Thus at the dosage employed ferrous iron supported a higher hemoglobin level for infants than did ferric iron.